OBSERVATIONS

ON THE

GROWTH AND REPRODUCTION

OF THE

RED CORPUSCLES OF THE BLOOD.

BY

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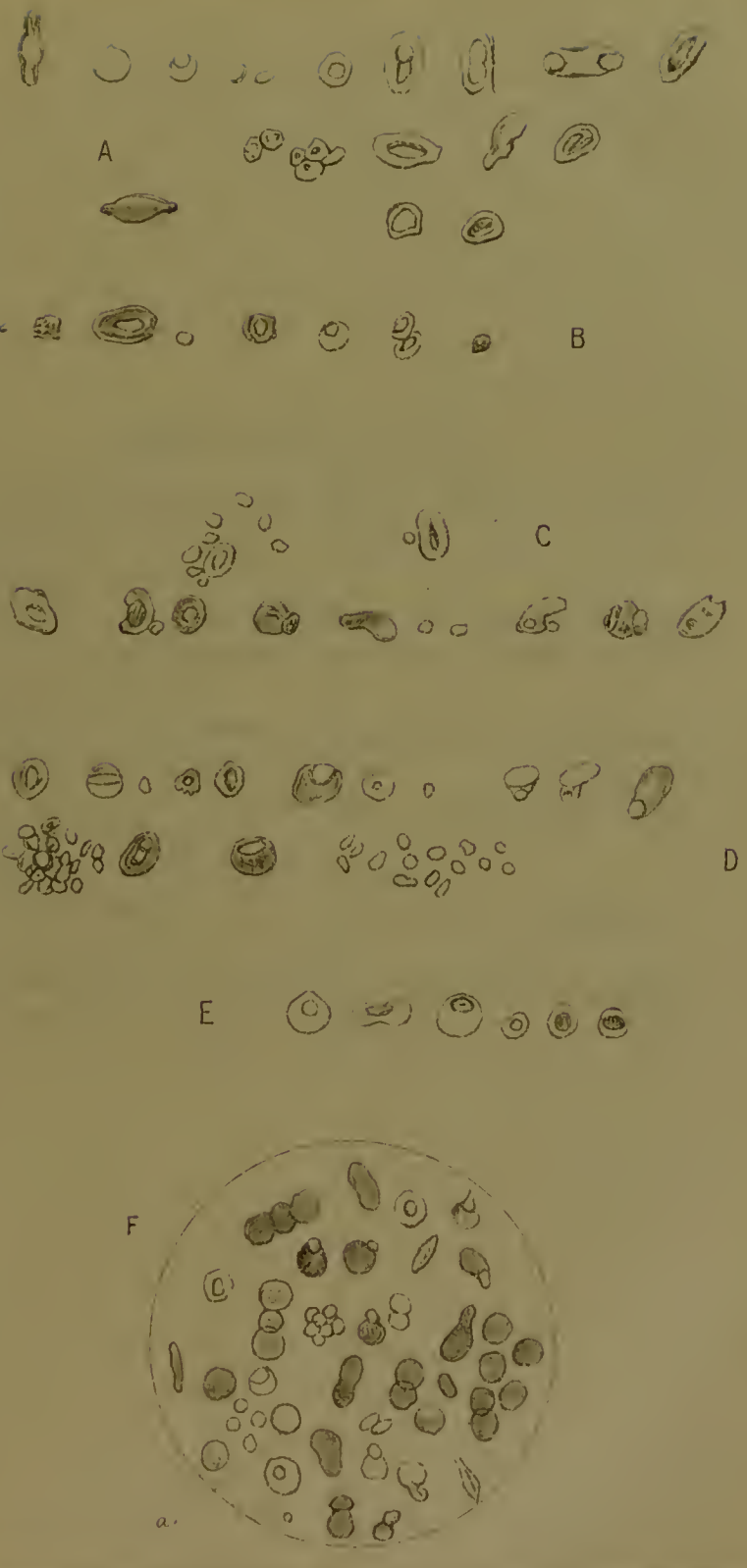
A
B
C
D
E
F
a.

EXPLANATION OF PLATE.

A. Blood of goose mixed with the albumen of a hen's egg, and kept at the normal temperature of the human body for 8 hours.

B. Hen's blood and albumen of hen's egg whipped up with air, then kept in a closed tube for 8 hours at the temperture of 98.6 F (36.4 C.)

C. Blood of goose mixed with albumen of hen's egg, kept at 98.6 F for twenty hours.

D. The same kept for same period in about six times the bulk of its own serum.

E. D treated with dilute acetic acid.

F. Hen's blood kept for about 48 hours in albumen of hen's egg, at 98.6 F.

All the above were drawn from the specimens actually under examination, magnified 550 diameters.

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But little is known respecting the mode of growth and reproduction of the corpuscles of the blood. The recent discovery of nucleated red corpuscles in the marrow of mammalian vertebrates has thrown some light on the subject, but it is only necessary to read the numerous and conflicting hypotheses as to their formation, which may be found in even the most recent compendia of physiology to show that nothing certain is known of the matter. The red corpuscles of mammalia are somewhat of an anomaly in the body. Bearing the outward form of cells, having many of the properties of cells, yet destitute of a nucleus, and springing into being as it were without any certain parentage, they have always been the *crux* of physiologists. The writer was led to think that some knowledge of their mode of origin and reproduction might be obtained by growing them artificially, from the results accidentally obtained in the course of observations made on the virus of small-pox in the year 1871-72. The writer was at that time Physician to the Smallpox Hospital in Port of

Spain, Trinidad, and having taken up his residence at the Hospital for the purpose of better investigating the pathology of the disease, was in the habit of collecting the matter from the varioles in capillary glass tubes, such as are commonly employed for vaccine lymph. These, when filled, were taken to the writer's room for microscopical examination. It was not long before he observed a marked difference between specimens taken from the same patient, at the same time, according as they were examined immediately, or after an interval of a few hours. It was found that *up to a certain point*, the same series of changes which the matter undergoes when in contact with the patient's body in the variolous vesicle would take place in a sealed tube in his room. This room was in a roughly built wooden house exposed without shade to the tropical sun, and having an iron roof. The temperature was generally at 90° nearly all day, and rarely below 70° all night. Some account of these experiments will be found in papers on the "Pathology and treatment of Smallpox," published in the 'Medical Times and Gazette' of 1872, and reprinted as a pamphlet. The subject has engaged the writer's attention, and he has been engaged in experiments with vaccine matter and pus at intervals ever since. As these experiments are still incomplete, it would be premature to say any more about them, than that they satisfied him that not only cells from the vaccine pock would live and grow in the serum of blood, but that the red corpuscles of the blood itself would grow and reproduce themselves outside the living body, under favorable conditions, and if supplied with a suitable pabulum. These experiments were made in the following manner. A small clot from fresh blood which had coagulated sufficiently long for some serum to exude, was placed in a small glass bottle. The clot usually occupied from 1-5th to 1-6th of the bottle, the remainder was filled by the serum of the same blood, and the bottle care-

fully closed so as to leave no air between the cork and the contents. Particular attention was paid to this point at first, from the fear that putrefaction would occur if air were allowed to enter. It has since been found that a small quantity of air does not produce putrefaction, and that the bottles may be opened again and again, and their contents examined without any fear of such a result. The larger sized vaccine tubes may also be used. The first effect of putting the serum on the clot was that the former was immediately reddened by the diffusion of red corpuscles through it And it will be found that serum thus reddened if put in a cold place requires several hours—12 at least—before it becomes perfectly colourless again. The necessary movements of corking the bottles, &c., invariably produce a deep reddening of the serum. The first change perceptible to the naked eye when the bottles thus charged have been subject for about three hours to the heat of the human body is that the red corpuscles separate very readily from the serum, and form a stratum at the most depending part of the bottle. At the same time they lose their red tint and become lake coloured. If shaken up so as to mix intimately with the serum, they very quickly fall down again, and in a few minutes the serum is quite clear of them—as far as the naked eye can see. It looks tinted but is not red. Thus it is evident that the red corpuscles *have changed their colour*, and have become relatively to the serum, of a greater specific gravity, or they would not so readily subside. It is not established whether the serum has diminished in gravity or the corpuscles have gained. One thing is certain, that they subside very quickly.

While these changes are going on, some kind of gas is formed, as however carefully the air may have been excluded, a small bubble is always found. In one series of experiments in order to make certain that no air had got in accidentally

the tubes were closed with melted wax, but the air bubble always appeared. Under the microscope it was apparent that "the Red Corpuscles were dividing by budding and fissure. Many are elongated and oval, others are nearly divided; there are many small circular bodies of same colour as the red corpuscles but much smaller." This is copied from notes taken at the time.

The next day another specimen kept under the same conditions is noted as having "great diversities in size of the red corpuscles, but, as a rule, much smaller." The next day it is noted that "there are still red corpuscles dividing, and many in pairs, as if not yet separated. A number of pale cells like decolourized red corpuscles. All these disappear with acetic acid."

These notes were made primarily with reference to the reproduction of vaccine and pus, and with no intention of experimenting on the red corpuscles, which had got in accidentally. About this time having under his care some cases of croupous pneumonia the writer examined the rusty coloured sputa, and then found that in specimens distinctly coloured to the naked eye, there was either very few, or, in some cases, no normal red corpuscles, but many presenting various irregular forms and especially many small spheroidal bodies of the colour of blood corpuscles, and similar to those noted above.

These observations coincided with those made by Beale, as far as the changes in form go, but he does not speak of them as occurring out of the body.

In the sputum of pneumonia many rows of red corpuscles will be seen, somewhat altered in shape, generally much swollen, and arranged like a string of beads. These are often pulled out by the mucus, in which they are entangled. If this mucus be submitted to the heat of the human body for a few hours, in place of these strings of red corpuscles there

will be found similar collections of much smaller bodies, evidently occupying the place of the red corpuscles, which have disappeared. This experiment must be very carefully performed, as sputum detached from the body, but kept at a temperature of 98.6, will putrify in about 6 hours, and gives off a most horrible smell. Apparently this is from the mixture of saliva, as mucus hawked up from the throat direct, without touching the mouth, keeps for a much longer time—twenty hours in fact. Possibly the presence of the sulphocyanides in the saliva may favour rapid decomposition.

These experiments, of which the details would be tedious, taken together with the fact that the red colouring matter of the blood is diffused through pneumonia (and some other sputum) in a way which never could take place mechanically, convinced the writer that some vital changes would occur in blood removed from the blood-vessels, but supplied with pabulum, and kept at a temperature of 98.6.

On thinking the matter over it occurred to the writer that the nucleated oval corpuscles of birds would be not only much larger, but would show much more clearly the nature of the changes that took place. By this means several inconveniences attending the use of mammalian blood—such as the extreme smallness of the corpuscles—were avoided, but as it was difficult to procure enough serum from the blood itself, in many of the experiments the albumen of the hen's egg was employed, which answers just as well as serum. The first thing to be noted is that however soon the blood may be examined after being taken from the bird, it will be found to contain not only oval nucleated red corpuscles and circular white ones, but also non-nucleated circular red corpuscles exactly like those of the mammalia, except that they appeared to be biconvex. These have been seen within fifteen minutes of the blood being taken from the body. It is important to note

this fact, as it bears out and explains the changes that subsequently take place.*

The first of these is that the corpuscles become thicker, and the nucleus swells. This is seen very well when the corpuscles turn on their edges. Many of them then or soon after become bent or curved, so that their outline when they turn on their side is that of a concave convex lens. (See Plate A.) Numerous very minute, bright, highly refracting spherical particles are seen. These do not coalesce, and are nearly all of one size. At the end of an hour's exposure to the heat of the body, it was observed that many of the round red corpuscles (which when the blood was first drawn presented no appearance of a nucleus) showed nuclei; a few oval corpuscles had serrated edges. No change in the great majority.

At the end of 24 hours, the "red corpuscles had precipitated in the manner previously described; the albumen was much more liquid; nuclei becoming large, round, and well defined; many circular corpuscles."

The next change observed is that the shapes of the corpuscles are now much altered, and may be pyriform. Many of the nuclei are double, and are evidently about to divide. Their mode of division is a subsequent stage. They separate widely within the corpuscles, one going to one side or end, and the other to the opposite. (Plate A.) In some cases it appears as if the germinal matter divided into four parts, The nucleus becomes elongated and narrow in some cases, and in others spherical and filling out the corpuscles. The nucleus escapes leaving a gap in the outer part of the corpuscles, which then rapidly shrinks and loses its colour. In subsequent stages it may be noted that the old corpuscular walls are often seen massed together, quite colourless, and

* Beale also describes in human blood the appearance of red corpuscles of very varied size and shape. I have described some of these varieties, especially the serrated edges, in a paper read before the Anthropological Society, and published in their Journal, "On the appearances presented by the red corpuscles of the blood in various races of mankind." Rollet (New Sydenham Society's Translation of Stricker's Histology) also describes several varieties of the red corpuscles.

their outlines only to be seen by a careful management of the light, and granular, as if undergoing fatty degeneration. After being kept all night in contact with the body the large majority of the oval corpuscles have disappeared; those that remain are much changed in outline, and evidently about to proliferate, while a number of free nuclei are seen, sometimes singly, but usually massed together in groups. (Plate B.) At first these nuclei are simply granular, and either round or oval; there are also many circular bodies containing no apparent nucleus.

Some specimens will be found to contain small circular bodies, very transparent, with a dark nucleus in them. These are often grouped together in twos and threes, and sometimes in larger numbers. They are of a size intermediate between that of the free nuclei and that of the red corpuscles, and are evidently nuclei taking upon themselves a cell-wall and contents. In all specimens there are a large number of the brightly refractive particles before spoken of.

If kept for two nights in contact with the body, being exposed during the day to the ordinary temperature (about 65°), it will be found that every oval corpuscle has disappeared. The entire precipitate is composed of the following elements: circular nuclei in immense numbers grouped together, and forming a jelly-like mass in the albumen. To the naked eye the appearance is very much that of the spawn of some of the fresh-water mollusks. Some of these nuclei are slightly granular, and nearly opaque; mostly, however, they have a very distinct outline, and are transparent. In many may be seen, by a careful management of the light, a cell-wall forming round them, at first barely perceptible, and little larger than the nuclei. But others are found with a more distinct and larger cell-wall, and a considerable number are well-defined transparent cells with a highly refracting nucleus. In the latest stage to which their development can be carried

before putrefactive changes occur, the nuclei have become converted for the most part into circular bodies of a fawn colour, like that of red corpuscles, some nucleated, some not, some spherical or nearly so, others thick disks. Some of these are elongated or pyriform, others apparently budding. With these are found groups of nuclei, and some of the transparent nucleated cells which have not yet taken to themselves the colouring matter of the blood. It seems as if these circular fawn coloured bodies would under favouring circumstances develop into the regular oval corpuscles of the blood, as they have a tendency to assume that shape. See Plate F.

The steps of the process seem to be as follows :—

1. Swelling of the whole corpuscle, but especially of the nucleus.

2. Elongation of the nucleus, and commencing division into generally two, but sometimes four parts.

3. Division of the nucleus, and separation of the parts within the wall of the corpuscle.

4. Rupture of the wall and escape of the nuclei.

5. Shrinking, and ultimate fatty metamorphosis of the corpuscle.

6. Agglomeration of the free nuclei into groups or masses.

7. The nuclei take on them a transparent jelly-like envelope, which

8. Enlarges until it assumes the form of a distinct cell-wall with contents, and

9. Absorbs the colouring matter of the blood, while the nucleus becomes obscured, but may be brought out by dilute acetic acid or water.

To show that these changes are really vital, it is worthy of note that the same blood which had been thoroughly dried, and another portion which had commenced to putrify were mixed with albumen, and subject to the heat of the body, and in both cases nothing but the ordinary putrefaction changes took

place, attended with much fœtor, and the growth of multitudes of vibriones, bacteria, &c., &c.

Being desirous of ascertaining the effect of a fever temperature on the growth and development of the red corpuscles, some hen's blood was subjected to the temperature of 104 deg. 5 sec. F; most of the time the latter, but never exceeding 105 deg. For a short time the temperature fell to 102 deg., owing to the room having got cold. The blood mixed with albumen of hen's egg was placed in small (½ drachm) corked bottles in a water bath which was kept warm by means of a lamp. These experiments need not be given in detail, it may suffice to say that while a portion of the same blood and albumen subject to the heat of the human body (98.6 F) went through the regular series of changes, that subjected to the fever heat, hardly underwent any change at all for six hours, when the experiments were given up.

It was curious that this same blood which had been subjected to the high temperature, after remaining a night and a day in the cold, went through all the ordinary changes enumerated above when put in contact with the human body, showing that though the high temperature retarded metamorphosis it did not kill the blood. Further experiments will be made on this point.

A specimen of mixed blood and mucus instead of putrefying as it usually does in a few hours, and giving out so much gas as to blow out the cork and produce a most offensive smell, remained fresh for the whole night. In every other case 4 or 5 hours has been sufficient to produce putrefaction.

It is also worthy of note that these specimens have been kept at the heat of the human body for 24 to 30 hours, and then at the ordinary temperature of some hot summer days without putrefaction. Yet it is well known that animal substances exposed to a heat of less than 90 deg. will putrefy in

from 12 to 16 hours. But as long as these vital changes are taking place putrefaction does not occur.

There are many interesting pathological questions which may probably be elucidated by this new method of investigation; for it seems to have occurred to none of the eminent men who have employed themselves in researches on the physiology and pathology of the blood, to keep it at or near the temperature of the human body in health and disease. Very high temperature, such as 110-15 has been employed, but never so far as the writer can discover, a continuous heat of 98.6.

The most convenient method of experimenting is to get some of the small bottles used by homœopathic chemists for their globules, and fill them with the mixture of blood and serum or albumen. By filling them quite full and then corking very slowly and carefully all air may be excluded. They should then be stitched into a long piece of calico, and tied round the body, under all the clothes. The larger vaccine tubes have also been employed. They can be kept in a case, and one taken out at intervals for examination. There need be no hurry to use the blood immediately after the bird is killed, as the blood retains all its vital porperties for twelve hours, in a temperature under 70 F.

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